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Screening of the kernels of *Pentadesma butyracea* from various growing sites of Benin and evaluation of their antioxidant pigments content

Bernolde P. Ayegnon[1], Adéchola P. P. Kayodé[1,2*], Glawdys Gnanvi[1], Yann Madodé[2], Fagla-Amoussou Balbine[1,2], Paulin Azokpota[2,3], Mohamed M. Soumanou[4] and Joseph D. Hounhouigan[2]

[1]Laboratoire de Valorisation et de Gestion de la Qualité des Bio ingrédients Alimentaires (LABIO) ; Faculté des Sciences Agronomiques ; Université d'Abomey-Calavi; 03 BP 2819 Jericho Cotonou, Bénin.
[2]Laboratoire de Biochimie Microbienne et de Biotechnologie Alimentaires (LMBA), Faculté des Sciences Agronomiques, Université d'Abomey-Calavi; 03 BP 2819 Jericho ; Cotonou, Bénin.
[3] Laboratoire de Biologie Moléculaire et Formulations des Aliments (LAFAB) ; Faculté des Sciences Agronomiques, Université d'Abomey-Calavi; 03 BP 2819 Jericho Cotonou, Bénin.
[4]Laboratoire d'Etude et de Recherche en Chimie Appliquée (LERCA), Unité de Recherche en Génie Enzymatique et Alimentaire ; Ecole Polytechnique d'Abomey-Calavi, Université d'Abomey-Calavi; 01 BP 2009 Cotonou, Bénin.

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***Pentadesma butyracea Sabine* (Clusiaceae) is a ligneous forest species of multipurpose uses. It is widely distributed in Africa from Guinea-Bissau to the West of the Democratic Republic of Congo. This study screened the kernel of *P. butyracea* on the basis of their physico-chemical properties. Six types of kernels were distinguished. The plant producing type 1 kernels (with medium length, low width and high thickness) and type 5 kernels (with high length, high width and high thickness) predominate within accessions representing 63% of the collections. Significant differences were found in the structural composition of the kernels. The average level of oil concentration in the kernels was 46.77%. The 1000 kernel weight averaged to 6.46 kg. The kernel hardness averaged to 550.81 N and is similar for all accessions but revealed that *P. butyracea* kernel is harder than shea kernel. The total phenolics and the total anthocyanins contents of the kernels varied significantly among accessions with a mean value of 164.03 and 152.78 mg/100 g, respectively. The antioxidant activity in the extract from *P. butyracea* kernel ranged from 30.22 to 58.57% of the remaining 2,2-diphenyl-1-picrylhydrazyl (DPPH) and is comparable to many traditional sources of antioxidants.**

Key words: *Pentadesma butyracea*, kernel, butter, antioxidant, cosmetic.

INTRODUCTION

Forests Galleries play a significant role in the socio-economic and cultural life of the populations in the Sudanese area of West Africa (Natta, 2007; Ceperley et al., 2010). These forests shelter a wide diversity of plant species. Forests Galleries of Benin contain approximately 1000 species of plants, which represent approximately

the third of the total flora of the country (Natta, 2003). This phytodiversity includes a wide range of multipurpose tree species among which *Pentadesma butyracea Sabine* is of prime importance (Natta et al., 2011). The multipurpose character of *P. butyracea* derived from the various usages of its different organs, that is, seeds, leaves, flowers, bark and roots. These organs are used in food, cosmetic and therapeutic applications (Abbiw, 1990). In Gabon and Côte d'Ivoire, the marcerated bark is utilized in lotions for treatment of the skin parasitic diseases and as antidiarrhoeatic (Raponda-Walker and Sillans, 1961). In Ghana, the decoction of the roots is used to fight intestinal worms (Abbiw, 1990). Recent works on the biological activities of *P. butyracea* showed that the xanthone isolated from their roots and stem bark present some antiproliferative, cytotoxic and anti-plasmodial activities (Zelefack et al., 2009; Wabo et al., 2010). Many authors even reported about the economic, nutritional, social and cultural significance of *P. butyracea* (Dencausse et al., 1995; Sinsin and Sinadouwirou 2003; Tchobo et al., 2007; Avocevou-Ayisso et al., 2009, Natta et al., 2010). The fruits of *P. butyracea* are ellipsoid, pointed, and are about 15 cm long and 10 cm large (Hutchinson and Dalziel, 1954). They contained oleaginous seeds which are consumed like kola (Sinsin and Sinadouwirou, 2003) and from which an edible butter is extracted (Mabberley et al., 1987). As much as 25% of oil can be extracted from the kernel of *P. butyracea* using the traditional extraction procedure.

Fundamentally, the butter of *P. butyracea* resembles the sheanut (*Vitellaria paradoxa)* butter in many aspects, but it possesses superior quality which confers to the butter a wide range of functional attributes (Ayegnon et al., 2015). Thus, the butter of *P. butyracea* is extensively used in traditional medicine as massage oil, in skin and hair care, and in the soap manufacturing because it possesses softening, lubricating and healing qualities (Dencausse et al., 1995). Interestingly, the butter of *P. butyracea* was used to retard the ageing of skin in patented cosmetic preparation (Courtin, 1986). So far, the processing of the *P. butyracea* kernels into butter is artisanal and rather a tedious activity done by rural women (Sinsin and Sinadouwirou, 2003).

Basically, the butter extraction from the *P. butyracea* kernel involves the seeds boiling, drying, roasting, crushing in mortar with pestle, grinding in a disc mill or millstone, churning to generate a cream which is cooked to obtain the oil. Crushing is the most difficult unit operation in the process due to the kernel hardness. In view of mechanizing this unit operation, important prelim-nary data should be generated on the physicochemical properties of the kernel. Indeed, many seed characteristics such as structure, sharp, weight, hardness and colour are useful in the designing and choice of the processing equipments of plant seeds. In the present study, we collected seeds from 30 accessions of *P. butyracea* in different agroecological zones of Benin and characterized them for their physical properties including 1000 kernel weight, dimension, sharp and colour characteristics of the kernel. As important by-products such as oilcake are generated during the kernel processing, their valorisation can increase the economic value of the kernel of *P. butyracea*. Thus, we extracted and quantified the phenolic pigments as well as their antioxidant capacity in the whole kernel and its various compartments using hydrophilic extraction procedure. The antioxidant pigments have great potential in health promoting applications. Also, they are used in food products to delay or inhibit the oxidation process and to increase the product shelf life and quality. In many cases, antioxidants are primarily added to foods in combination with synergists like ascorbic, tartaric and phosphoric acids to increase efficiency (Pietta, 2000). Currently, cheap sources of these components are searched for.

MATERIALS AND METHODS

Seed collection

The seeds of *P. butyracea* were collected from May to June 2013 in the forests galleries of four communities in northern Benin that is Tchaourou (8°45'-9°20' N and 2°10'-3°40' E), Kandi (11° 7'- 11°43' N and 2° 56'-2°13' E), Toucountouna (10°20′ - 10°45′ N and 1°10′ - 1°40′ E) and Bassila (8°30′ - 9°30′ N and 1°00′ - 2°30′ E). These collection sites belong to various agroecological zones of Benin. Thus, Tchaourou and Bassila are localised in the Sudano-Guinean transitional climatic zone with an average rainfall varying from 850 to 1850 mm and 1000 to 1300, respectively. Kandi and Toucountouna are located in the Sudanian climatic zones with an average rainfall of 1200 and 1000 mm, respectively (Assogbadjo et al., 2005). Riparian forests with abundant *P. butyracea* are common along rivers in these different sites. We adopted the transect method during the seed collection, maintaining a distance of 30 to 50 m between two consecutive trees to prevent gene flow engendered by pollinator agents. Fruits were collected from 30 accessions of *P. butyracea* and depulped on the field. The extracted seeds were packed in jute bags and transported to the laboratory. Kernels were washed and dried at 45°C for 72 h before analysis. For chemical analysis, samples were ground into powder using an ultra-centrifugal mill (Retsch GmbH, Haan, Germany) with a 1 mm sieve and subsequently stored at -20°C until use.

Determination of the structural composition of the seed

The structural composition of the kernel was determined by separating the different compartments of the kernel, that is, the germ, albumen and tegument. Subsequently, the percentage of each part was calculated on the basis of the kernel total weight on dry matter basis. Measurements were performed in duplicate on 10 kernels chosen at random per accession.

*Corresponding author. E-mail: polykap@yahoo.fr. Tel: (+229) 97870734.

Determination of seed shape

The kernel dimensions that is length, width and thickness were measured with a precision caliper (0.01 mm). Measurements were performed on 100 kernels chosen at random per accession. The generated values allow calculating the various shape characteristics of the kernels such as elongation and degree of flattening using the following formulas (Zavrajnov and Nikolow, 1990):

Elongation of the kernels = L/l; Degree of flattening = l/e; where L= length, l= width and e= thickness

Determination of 1000 kernel weight

The 1000 kernel weight was determined by weighing 100 grains of each sample on a 1/10.000 precision balance and multiplying the obtained value by 10. The weight was expressed on dry matter basis. Measurements were performed in duplicate.

Measurement of the kernel hardness

The kernel hardness was determined by measuring its minimal strength of resistance to the bruising using a texturometer LF Plus (LLYOD Instruments, USA) fit with a 0.42 cm thick blade with a triangular cover of Warner Bratzler type. Measurements were replicated 5 times and average values were reported.

Colour measurement

Colour measurements were performed using Konica Minolta chromameter (INS CR410, Japan). Results were expressed as L* (brightness) and a* (redness). The colour coordinates of the white ceramic standard are:

Y = 86.10, x = 0.3194, y = 0.3369.

Determination of fat and ash contents

Fat was extracted from 40 g of ground kernels with hexane using a Soxhlet extractor (AOAC, 2002). The fat content was gravimetrically measured after removal of the solvent by rotary evaporation under vacuum. Extraction was run in triplicate on germ, albumen and whole kernels. Total minerals content was determined following the standard AOAC methods (AOAC, 2002) and calculated in percentage of dry matter.

Preparation of extracts for the determination of phenolics, anthocyanins and antioxidant capacity

Samples were extracted in methanol/HCl (85:15 (v/v) following the method described by Kayodé et al. (2007). Fifty milligrammes (50 mg) of each sample were extracted at room temperature with 1.5 mL of solvent under agitation using a magnetic stirrer for 30 min. The mixtures were centrifuged at 2500 *g* for 10 min and the supernatants were collected. The residues were re-extracted twice under the same conditions, resulting in 3 mL crude extract. All extracts were used as they were after centrifugation for various analyses.

Total anthocyanins determination

Total anthocyanins content was calculated as described by Abdel-Aal and Hucl (1999) using cyanidin 3-glucoside as standard pigment. The absorbance of the pooled extracts was measured after centrifugation at 525 nm against a blank reagent. Total anthocyanins content was expressed as mg cyanidin 3-glucoside equivalent per 1 g powder based on DM.

Total phenolics determination

Total phenolics were measured following the method of Singleton and Rossi (1965) modified by Kayodé et al. (2007) as follows: to 300 µL of extract, 4.2 mL of distilled water, 0.75 mL of Folin-Ciocalteu's reagent (Merck, Germany) and 0.75 mL of sodium carbonate solution (200 g L^{-1}) were added. After incubation for 30 min, the optical density was measured at 760 nm against a blank. Gallic acid was used as standard and the results were expressed as gallic acid equivalent (GAE) per g of sample DM.

Determination of antioxidant capacity

Extracts obtained with aqueous methanol were analyzed for their total antioxidant capacity by evaluating the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging ability of sample extracts. DPPH radical-scavenging activity of sample extracts was determined according to the method reported by Brand-Williams et al. (1995). The reaction mixture consisting of 1.5 mL of DPPH working solution (4.73 mg of DPPH in 100 mL of HPLC-grade ethanol) and 300 µL sample extract was shaken and incubated for 40 min in the dark at room temperature. The absorbance was measured at 515 nm against a blank, using a UV-vis spectrophotometer (6715UV/Vis. JENWAY). DPPH free radical-scavenging ability was calculated using the following formula:

Scavenging ability (%) = [Absorbance $_{515\ nm\ of\ control}$ – Absorbance $_{515\ of\ sample}$/Absorbance of control] x 100

Data analysis

The seed dimension characteristics were subjected to a hierarchical ascending classification using the SAS 9.1 program. This allows to group similar length, width and thickness. A discriminate canonical analysis was also performed on the identified groups to test and validate the differences. The ANOVA model was used to compare means between groups with the Duncan post-hoc test and significance level set at $p < 0.05$. Principal component analysis (PCA) was performed for cluster analysis.

RESULTS AND DISCUSSION

Typology of the *P. butyracea* kernels

Three thousands (3000) of kernels from 30 accessions of *P. butyracea* (100 kernels per accession) collected in four communities of Benin were characterized for their length, width and thickness (Table 1). The length of the kernels ranged from 3.13 to 4.14 cm, with a mean of 3.58 cm and their width ranged from 2.24 to 3.01 cm, with an average of 2.73 cm. The thickness of the kernels averaged was 2.26 cm. The mean values of the elongation and flattening of the *P. butyracea* kernels are 1.34 and 1.22, respectively. The kernel of *P. butyracea* is bigger than shea kernel. Indeed, various dimension related data

Table 1. Typology of the kernels of *P. butyracea* based on kernel sharp.

Kernel characteristic	Type 1	Type 2	Type 3	Type 4	Type 5	Type 6	
Length (cm)	3.33	3.42	3.54	3.09	4.33	3.95	
Width (cm)	2.38	3.11	2.96	2.44	3.17	2.64	
Thickness (cm)	2.44	2.60	2.22	1.91	2.47	2.06	
Kernel type							
Accession codes	**Proportion of each type in a total of 100 seeds from each assertion**						**Total**
P1	33	20	22	5	8	12	100
P2	47	15	15	7	16	0	100
P3	36	9	18	27	0	10	100
P4	0	0	0	11	34	55	100
P5	20	0	3	51	2	24	100
P6	31	2	13	26	6	22	100
P7	5	0	11	40	0	44	100
P8	6	0	0	85	0	9	100
P9	18	11	25	14	19	13	100
P10	15	14	11	10	36	14	100
P11	27	7	20	8	28	10	100
P12	33	24	17	12	9	5	100
P13	42	22	13	15	3	5	100
P14	16	13	26	10	16	19	100
P15	38	3	7	36	2	14	100
P16	7	14	8	24	31	16	100
P17	10	21	10	30	19	10	100
P18	0	20	29	0	20	31	100
P19	17	13	13	14	32	11	100
P20	11	7	14	1	49	18	100
P21	41	5	14	25	4	11	100
P22	22	0	18	42	0	18	100
P23	30	5	11	27	2	25	100
P24	32	20	17	13	10	8	100
P25	28	3	19	30	6	14	100
P26	10	10	20	10	35	15	100
P27	21	4	19	25	4	27	100
P28	13	4	23	21	31	8	100
P29	15	10	24	14	25	12	100
P30	24	16	26	13	11	10	100

reported for sheanut (*Vitellaria paradoxa)* samples collected in Benin and Burkina-Faso are lower than values found for *P. butyracea* kernels in this study (Ahouansou et al., 2008; Yé and Destain, 2004). Based on their length, width and thickness, the three thousands (3000) kernels from 30 accessions were classified into six distinct types of seeds using SAS software. The related coefficient of determination was significant (R^2 = 0.54) and is enough to obtain distinct classes of seeds. Thus, the type 1 seeds are represented by seeds with a medium length, low width and high thickness (Table 1). These seeds are thicker than larger. The accessions P_1, P2, P3, P11, P12, P13, P15, P21, P23, P24 and P25 are type 1 seed producing plants. The Type 2 seeds are characterized by a medium length, high width and a high thickness. Accessions producing this type of seed are

rare within the collection studied and none of the accessions could be classified as type 2 seed producing plant. The type 3 seeds include seeds with medium length, medium width and thickness. The accessions P9, P14 and P30 yield this type of seeds. The type 4 seeds are the smallest seeds with low length, low width and low thickness. The type 5 seeds represent the biggest seed category characterized by a high length, a high width and a high thickness. Finally, the type 6 seeds are distinguished by a high length, a medium width and a low thickness. The accessions P6, P7, P8, P17, P22 and P27 are assigned to type 4 seeds producing plants and the accessions P4, P10, P16, P19, P20, P26, P28 and P29 fall within the type 5 seeds. The accessions P5 and P18 produce type 6 seeds. Clearly, there is a big variation within the kernels of *P. butyracea* in terms of their morphological characteristics. The major factors that could affect these characteristics are the plant age, its genotype and its growing environment (Salazar et al., 1987). From the above classification, it appears that the plant producing type 1 and type 5 seeds predominate within the accessions representing 63% of the collections studied. The plant producing types 4 seeds account for 17% of the accessions and those producing type 3 and 6 seeds represent 10% each. The present classification offers quantitative data on the morphology of the *P. butyracea* seeds which can be helpful in the design of seed processing equipments.

Structural composition and physical properties of the *P. butyracea* kernels

The kernel structural analysis revealed that *P. butyracea* kernel is composed of a tegument (3.14% w/w), an albumen (56.32%) and a germ (38.45%) (Table 2). The growing site did not affect the structural composition of seed but significant differences were found between accessions for this parameter ($p \leq 0.0001$). More specifically, kernels from the accessions P17, P18, P22, P23 and P25 collected in Bassila contain relatively high proportion of albumen while the accessions P2 and P9 originated from Tchaourou and Toucountouna, respectively concentrate the lowest fraction of albumen in their kernels. The differences observed in the albumen fraction of kernel could be assigned to the genetic makeup as well as to the growing environment of the plant (Mathur et al., 2009). Mean values for the colour parameters of the kernels of *P. butyracea* by regions are presented in Table 2. All samples exhibited relatively high values for the redness index (a*) indicating that the *P. butyracea* kernel is a redish almond as previously reported (Aïssi et al., 2011).

There is a significant difference between the accessions studied for their kernel colour ($p < 0.001$). Thus, the accessions P5 and P10 sampled in Kandi and Toucountouna, respectively have a very red kernels (highest value of a*), while the lowest value of a* was recorded for kernels from accessions P1, P7, P18, P25, P26 and P29 from various localities. Low values of L* were observed in the accession P2, P3, P7,P17, P18 and P26 from Tchaourou, Kandi, Toucountouna, Bassila and the highest value was from the accession P4, P5 and P20 originated from Tchaourou, Kandi, Toucountouna and Bassila. Negative correlation were obtained between the albumen and the tegument fractions ($r = -0.268$; $p \leq 0.0001$) and between the germ and the albumen fractions ($r = -0.963$; $p \leq 0.001$). Likewise, the tegument fraction negatively correlated with the kernel L* and a* values ($r = -0.263$; and $r = -0.242$ at $p \leq 0.01$ respectively). A positive correlation coefficient was obtained between germ fraction and L* value ($r = 0.233$; $p \leq 0.01$). The 1000 kernels weight for the *P. butyracea* kernels ranged from 3.76 to 9.06 kg, with an average value of 6.46 kg (Table 2). The growing site did not affect the 1000 kernels weight but there is a significant difference between accessions for this parameter ($p \leq 0.0001$). Thus, the heaviest seeds were recorded in the accessions P6, P9, P10, P16 and P19 collected in Kandi, Toucountouna and Bassila, while the kernels with the lowest weight were found in accessions P7 and P8 that originated from Kandi. The kernels of *P. butyracea* are heavier than the shea kernels for which the mean value of 1000 kernels weight amount to 4 kg for samples collected in various areas of Benin (Ahouansou et al., 2008); and to 2.5 kg for samples collected in Burkina Faso (Yé and Destain, 2004).

Nevertheless, sheanut samples collected in Nigeria presented a mean value of 8 kg for their 1000 kernels weight (Aviara et al., 2000). Likewise the *P. butyracea* kernel is 1.5 times harder than the sheanut kernel (Table 2). However, there is no significant difference between accessions for their kernel hardness. Our data resemble the findings by Ahouansou et al. (2012) who reported values ranging from 582 to 611.84 N for the hardness of *P. butyracea* kernel collected in Benin. An important implication of this result is that the kernels of *P. butyracea* would possess high resistance to attacks by insects, mould and rodents; and would have a good storage behaviour. However, the design of seed processing equipments (crusher or grinder mills) may necessitate stronger materials.

Oil content of the kernels from different accessions

The oil and ash contents of the whole kernel of *P. butyracea*, its albumen content and germ fraction are presented in Table 3. The kernels oil content varies significantly between accessions and ranged from 43.90 to 49.55% with a mean value of 46.77%. These data resemble findings by Tchobo et al. (2013) and Aïssi et al. (2011) who reported values of 41.9 and 48.66%, respectively, for the oil content of the *P. butyracea* kernel.

Table 2. Structural composition and physico-chemical characteristics of the kernels *of P. butyracea*.

Collection sites	Structural composition (% of whole kernel)								Kernel colour	
	Tegument	Albumen	Germ	1000 kw (kg)	Kernel hardness (N)	Oil content (%)	Elongation	Flattening	Redness (a*)	Lightness (L*)
Tchaourou	2.89±1.13[a1]	52.76±5.36[a]	37.05±4.68[a]	6.19±0.57[a]	549.57±20.49[a]	45.29±0.76[a]	1.33±0.04[a]	1.16±0.04[a]	5.36±0.48[a]	43.20±0.87[a]
(n= 3)	[2.19-4.20]	[46.99-57.60]	[31.84-40.91]	[5.55-6.63]	[552.42-568.49]	[44.82-46.28]	[1.30-1.37]	[1.11-1.19]	[4.80-5.64]	[42.67-44.91]
Kandi	3.72±0.39[a]	53.51±3.52[a]	41.43±3.74[a]	5.87±1.81[a]	566.90±20.83[a]	45.32±1.08[a]	1.48±0.05[b]	1.25±0.06[a]	6.46±1.37[a]	44.67±1.68[a]
(n= 5)	[3.46-4.37]	[49.56-58.60]	[36.83-45.45]	[3.76-8.45]	[543.83-596.43]	[43.93-46.28]	[1.40-1.54]	[1.20-1.34]	[4.12-7.74]	[42.31-46.79]
Toucountou	3.17±0.58[a]	57.31±4.09[a]	38.06±4.35[a]	7.11±1.26[a]	548.40±18.53[a]	46.84±1.91[a]	1.29±0.06[a]	1.24±0.07[a]	5.78±0.68[a]	43.49±0.99[a]
(n= 10)	[2.56-3.99]	[50.00-64.82]	[29.38-45.37]	[5.52-8.61]	[521.20-576.43]	[43.94-48.93]	[1.21-1.37]	[1.12-1.33]	[4.84-7.05]	[41.59-44.68]
Bassila	2.92±0.56[a]	57.57±5.83[a]	37.88±5.96[a]	6.86±0.82[a]	546.44±20.62[a]	47.67±1.62[a]	1.33±0.05[a]	1.22±0.04[a]	5.84±0.76[a]	44.25±0.93[a]
(n= 12)	[2.32-4.19]	[48.10-68.44]	[26.57-47.51]	[5.52-8.11]	[512.80-568.54]	[43.90-49.55]	[1.24-1.35]	[1.20-1.29]	[4.49-6.92]	[42.75-45.55]
Average	3.83±0.65	57.01±5.05	39.14±4.97	6.46±14	550.81±20.28	46.77±7.83	1.34±0.08	1.22±0.06	5.88±0.85	43.96±1.15
CV [2]	13.31	4.13	4.36	12.46	1.49	2.53	5.18	2.45	6.65	1.32

[1]Mean ± Standard deviation; n = number of accessions included; values with the same letter in the same column, are not significantly different at p< 0.05. [2]CV= Coefficient of variation.

Table 3. Oil and ash contents and antioxidant pigments concentration in the different compartments of the *P. butyracea* kernels.

Kernel part	Oil content (%)	Ash content (%)	TPC (mg/100 g dw)	TAC (mg/100 g dw)	DPPH (%)
Albumen	37.49±7.73[a1]	2.19±0.35[a]	114.62±33.64[c]	119.28±26.10[a]	44.49±5.8[b]
(n= 30)	[26.90-38.68]	[1.67-3.03]	[70.71-181.97]	[73.25-192.53]	[32.39-57.13]
Germ	53.15±7.20[c]	2.82±0.40[b]	87.17±27.35[a]	123.78±30.34[a]	41.71±6.16[a]
(n= 30)	[41.78-65.77]	[1.80-3.49]	[52.12-175.55]	[71.02-190.65]	[30.22-53.87]
Whole kernel	46.77±7.83[b]	2.13±0.32[b]	164.03±31.54[b]	152.78±30.17[a]	48.21±7.11[b]
(n= 30)	[43.9-49.55]	[1.53-2.72]	[108.49-251.95]	[64.64-198.24]	[36.52-60.00]
CV [2]	17.19	16.06	31.95	13.78	7.27

[1]Mean ± Standard deviation; n = number of accessions included; values with the same letter in the same column, are not significantly different at p< 0.05. [2] CV= Coefficient of variation.

As reported elsewhere for *Vitellaria paradoxa* (Maranz and Wiesman, 2003; Di Vincenzo et al., 2005), the oil concentration in the *P. butyracea* kernel may be dependent of the genetic makeup of the crop as well as its growing environment. Also, immature fruits collected within sample may contribute to the kernel-oil variation (Harlan, 1975). The oil content of the albumen and germ fractions of the *P. butyracea* kernel averaged to 37.49 and 53.15%, respectively. As could be expected, the germ oil concentration is greater than that of the albumen. The *P. butyracea* kernel-oil content is comparable to that of sheanut kernel (*V. paradoxa)*. Crude oil content of shea kernel varies greatly according to authors. Value of 59.1% was reported by Tano-Debrah and Ohta (1994) while Nkouam et al. (2007) reported values ranging from 17.4 to 39.6 g/100 g dw using supercritical CO_2. According to Bup et al. (2012) the fat content of the kernel of *V. paradoxa* varies from 40 to 57% on the wet weight basis. The variation in the sheanut oil content was attributed to environmental influences as well as to the genetic diversity of the plant (Bup et al., 2012; Maranz and Wiesman, 2003; Di Vincenzo et al., 2005). The fatty acid profile of the *P. butyracea* oil is similar to that of sheanut and is mainly constituted of stearic (38 to 47%) and oleic (48 to 58%) acids which represented nearly 96% of the total fatty acids (Ayegnon et al., 2015, Tchobo et al., 2009). However, the *P. butyracea* butter possesses some superior qualities; being harder and having a much more pleasant aroma. Additionally, its natural bright yellow colour could represent an attractive characteristic on the butter market (Ayegnon et al., 2015). These differences can be used as comparative advantages in the design and formulation of new products. The ash content of the *P. butyracea* kernels (Table 3) is comparable to that of shea kernels (Duke and Atchley, 1986; Greenwood, 1929). The ash content of tegument is higher than that found in the albumen and germ.

Antioxidant pigments in the kernels of *P. butyracea*

Plant phenolics constitute one of the major groups of compounds acting as primary antioxidant or free radical

Table 4. Comparison of kernel of *P. butyracea* to other commodities for their antioxidant pigments contents

Commodity	Total phenolic (mg/g)	Total anthocyanin (mg/g)	DPPH (%)	Source
Kernel of *P. butyracea*	1.08 - 2.52[a]	0.65-1.98[d]	36.52 - 60.00% rem[i]	This work
Black rice	3.4 - 6.7[b]	1.1 - 2.6[d]	16.0 - 30.3% rem[i]	Sompong et al. (2011)
Red rice	0.8 - 6.9[b]	0.0 - 0.01[d]	13.0 - 62.8% rem[i]	Sompong et al. (2011)
Blueberry	2.2[c]	1.7[e]	16 µmol TE[j]/g FW	Dragović-Uzelac et al. (2009), Garzón et al. (2010)
Sour cherry	2.6[c]	1.9[e]	17 µmol TE/g FW[j]	Dragović-Uzelac et al. (2009)
Blackberry	-	6.6 - 9.2[d]	-	Cuevas-Rodríguez et al. (2010)
Black currant	-	0.28 [d]	-	Denev et al. (2010)
Red raspberry	1.49 - 3.48[c]	0.20[f]	-	Çekiç and Özgen (2008)
Strawberry	1.7 - 3.1[c]	0.10 - 0.30[g]	-	Tulipani et al. (2008)
Elderberry	-	0.63[d]	-	Denev et al. (2010)
Red cabbage	0.13 - 0.17[c]	0.60 - 0.85[e]	-	Podsędek et al. (2008)
Red onion	15.56[a]	0.45[d]	41.32 µMTE/g DM	Gorinstein et al. (2009)

[a]mg GAE/g of DM; [b]mg ferulic acid equivalent/g of DM; [c]mg GAE/g of FW; [d]mg C-3-glc/g of DM; [e]mg C-3-glc/g of FW; [f]mg C-3-soph/g of FW; [g]ACY based on HPLC data, mg/g of FW.

terminators. Many studies have related the phenolic contents of food products to their antioxidant activities, which are probably due to their redox properties (Chang et al., 2001b). Total phenolic content (TPC) of extracts from the different accessions was expressed as gallic acid equivalent (GAE) per g of DM. The TPC of the whole kernels of *P. butyracea* ranged from 108.49 to 251.95 mg/100 g dw, with an average value of 164.03 mg/100 g dw (Table 3). The TPC varied significantly between accessions ($p \leq 0.0001$). The highest values of TPC were found in samples P8, P17 and P20 from Kandi, Toucountouna and Bassila, while the lowest values were found in samples P6, P7, P9, P25 and P29 collected in Kandi and Bassila. The total anthocyanins content (TAC) of the *P. butyracea* kernels ranged from 64.64 mg/100 g to 198.24 mg/100 g dw with an average value of 152.78 (Table 3). The level of TPC in the albumen (114.62 mg/100 g dw) is higher than in the germ (87.17 mg/100 g dw) while no significant differences were observed between the albumen and germ for their TAC ($p \leq 0.05$). Positive correlation was observed between the TPC and the TAC of the kernels ($r = 0.267$; $p \leq 0.01$). Many authors investigated the phenolics content of shea kernel. Maranz and Wiesman (2003) reported that the gallic acid was the major phenolic compound of shea kernel and accounted for 27 to 70% of the measured total phenolics. The comparison of the *P. butyracea* kernel to other sources of antioxidants is presented in Table 4. The total phenolics content of the *P. butyracea* kernels is comparable to levels found in red raspberry, strawberry, blueberry and sour cherry but lower than values obtained in black rice (Sompong et al., 2011).

The total anthocyanins concen-tration in the kernel of *P. butyracea* is higher than values found in red rice, black currant, red raspherry, strawberry, elderberry, red cabbage and red onion, but lower than data reported by Cuevas-Rodríguez et al. (2010) for blackberry. The TAC in *P. butyracea* kernel is similar to levels found in blueberry, black rice and sour cherry. The total antioxidant capacity describes the cumulative capacity of food components to scavenge free radicals, and high intakes of dietary TAC have been related to several health benefits in both cross-sectional and randomized intervention studies. In this context, methanolic extracts were evaluated for their DPPH radical-scavenging activity. The remaining DPPH in the extract from *P. butyracea* kernel ranged from 30.22 to 58.57%. These values are higher than DPPH levels in black and red rice. Clearly, the *P. butyracea* kernel is a valuable source of antioxidant pigments that can be valorised to increase the economic value of this plant.

Cluster analysis

In order to better understand the relationship between the different accessions studied on the basis of the various physicochemical characteristics of the kernel, data were analyzed by principal component analysis (PCA). The physicochemical characteristics considered are: 1000 kernel, L*, b*, a* ΔE, germ, pericarp and albumen fractions, ash, total phenolics, anthocyanins and oil contents. On this basis, the first two main axes account for 83.50% of the total variation. The first principal component accounted for 46.9% of the total system variability and is positively correlated with the albumen fraction of the kernel, but negatively correlated with the seed colour characteristics notably with L*, a* and b* values, the seed germ, and anthocyanins contents. The second axe is positively correlated with 1000 kernel weight, the seed oil content and its albumen mass fraction; but inversely correlated with the kernel-germ and pericarp contents as well as its mineral concentration.

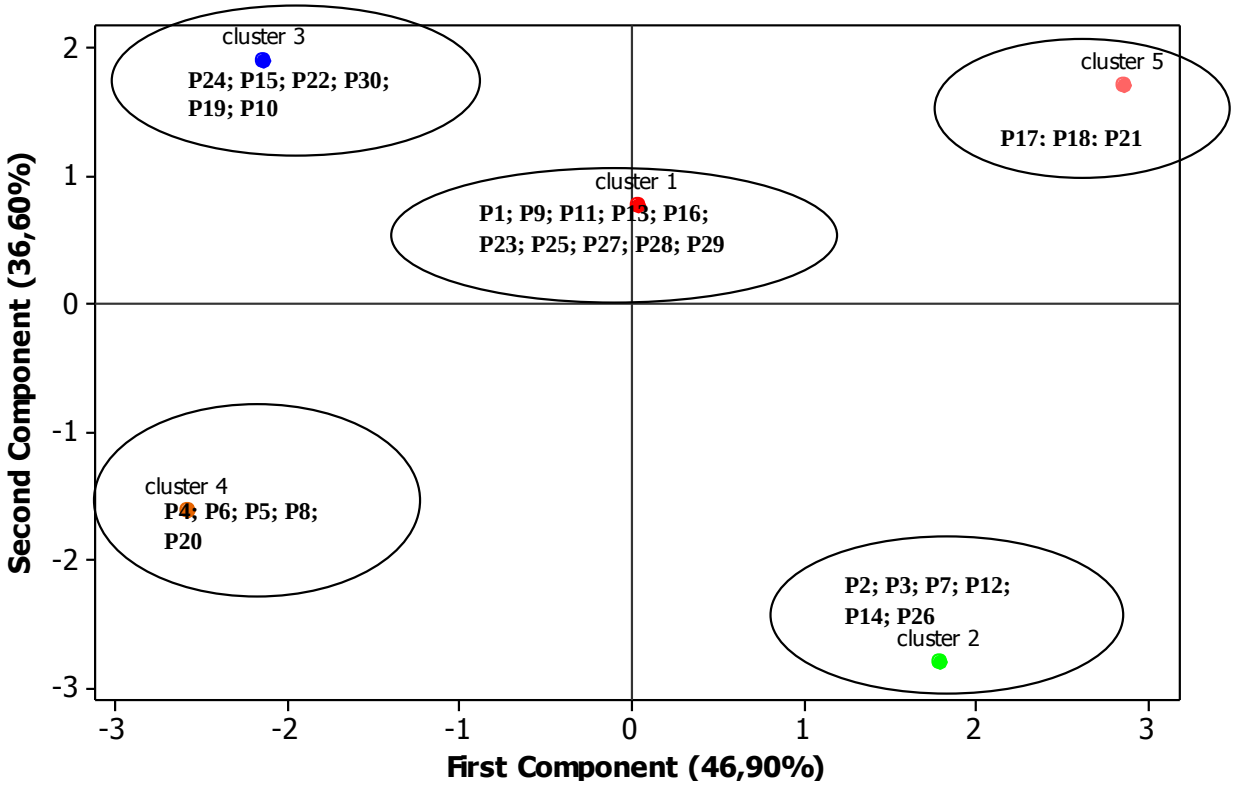

Figure 1. Clustering the different accessions of *P. butyracea* based on the physicochemical contents of their kernel.

The PCA allowed grouping the different accessions in five clusters (Figure 1). The first cluster is represented by the accessions P1, P9, P11, P13, P16, P23, P25, P27, P28, P29 which contain relatively low quantity of oil and germ in their kernel. But their anthocyanins content, mineral concentration and 1000 kernel weight are slightly high. These accessions come from Toucountouna and Bassila communities. The second cluster is located in the positive angle of the axis 1 and in the negative part of the axis 2. Six accessions, that is, P2, P3, P7, P12, P14 and P26 are grouped in this class. These accessions come from four communities in northern of Benin: Tchaourou, Kandi, Toucountouna and Bassila. Their seeds are character-rized by high values of colour parameters and high proportion of pericarp and germ in their kernel. But their oil content is slightly high and their albumen and anthocyanins contents are low. The cluster 3 is represented by the accessions P10, P15, P19, P22, P24, P30 which are characterized by high level of oil, anthocyanins content and albumen in their kernels but with low proportion of germ and pericarp in the kernel structure. These accessions are from Bassila com-munities. The accessions in cluster 4 (P4, P5, P6, P8, P20) contain low levels of oil and albumen fraction in their kernel with low values of colour parameters (L*, a* and b*). However, they possess relatively high proportion of germ, pericarp and mineral concentration. The majority of these accessions are originated from Kandi communities. Finally, the cluster 5 is constituted by the accessions P17, P18, P21 which are rich in oil, contain high proportion of albumen but possess low value for the ΔE and low proportion of germ and pericarp. Clearly, the accessions of *P. butyracea* from Toucountouna and Bassila municipalities are characterized by high level of 1000 kernel weight and anthocyanins content in their kernels compared to other accessions. The seed characteristics are likely to be determined by the growing environmental conditions as well as the genetic makeup of the plant.

Conclusions

The *P. butyracea* kernels vary in their physicochemical characteristics. The growing environment as well as the genetic makeup of the plant seems to affect the seed characteristics. The *P. butyracea* kernels are rich in antioxidant pigments. Particularly, levels of phenolics in the kernel are comparable to other traditional sources of antioxidants. This study provided useful data for the conception and the optimization of processing equipments for *P. butyracea* kernels. Further studies are needed to assess the impact of genotype and environmental conditions on the kernels and butter qualities.

Conflict of interests

The authors did not declare any conflict of interest.

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REFERENCES

Abbiw DK (1990). Useful plants of Ghana: African uses of wild and cultivated plants. Intermediate technology publications and the royal botanic Gardens, Kew, London. 337pp.

Abdel-Aal ES, Hucl P (1999). A rapid method for quantifying total anthocyanins in blue aleurone and purple pericarp wheat. J. Cereal Chem. 76:350-354.

Ahouansou RH, Aïssi MV, Sanya EA, Soumanou MM (2012). Physical and mechanical properties of *Pentadesma butyracea* seeds from Benin. J. Appl. Biosci. 50:3485-3493.

Ahouansou RH, Sanya EA, Bagan G, Foudjet A (2008). Study some physical characteristics of nuts and almonds of shea tree produced in Benin. J. Appl. Sci. Eng. Technol. 2:29-38.

Aissi M, Tchobo F, Natta A, Piombo G, Villeneuve P, Sohounhloue D, Soumanou MM (2011). Effect of the pretreatments post-harvest of the *Pentadesma butyracea* (Sabine) almonds on the technology of extraction in real medium and the quality of butter. OCL. 18: 384-392.

AOAC (2002). Official methods of analysis, 16 th edn. Washington, DC: Association of Official Analytical Chemists.

Assogbadjo AE, Sinsin B, Van Damme P (2005). Morphological characters and production of baobab tree capsules (Adansonia digitata L.) in Benin. Fruits 60:327-340.

Aviara NA, Haque MA, Izge IA (2000). Physical and frictional properties of sheanuts. Anal. Sci. Agro. 11:19-34.

Avocèvou-Ayisso C, Sinsin B, Adégbidi A, Dossou G, Damme PV (2009). Sustainable use of non-timber forest products: Impact of fruit harvesting on *Pentadesma butyracea* regeneration and financial analysis of its products trade in Benin. For. Ecol. Manag. 257:1930-1938.

Ayegnon BP, Kayodé APP, Tchobo FP, Azokpota P, Soumanou MM, Hounhouigan DJ (2015). Profiling the quality characteristics of the butter of *Pentadesma butyracea* with reference to shea butter. J. Sci. Food Agric. 10.1002/jsfa.7052.

Brand-Williams W, Cuvelier M, Berset C (1995). Use of a free radical method to evaluate antioxidant activity. LWT - Food Sci. Technol. 28:25-30.

Bup DN, Abi CF, Tenin D, Kapseu C, Tchiegang C, Moulougui Z (2012). Effect of cooking on moisture sorption isotherms of shea Nut (Vitellaria paradoxa Gaertn.) kernels PartII: Modelling and properties of sorbed water. Food Bioprocess Technol. 6:3273-3283.

Ceperley N, Montagnini F, Natta A (2010). Significance of sacred sites for riparian forest conservation in Central Benin. BFT 303:5-23.

Chang ST, Wu JH, Wang SY, Kang PL, Yang NS, Shyur LF (2001b). Antioxidant activity of extracts from Acacia confusa bark and heartwood. J. Agric. Food Chem. 49:3420-3424.

Courtin O (1986). Cosmetic preparation to retard the ageing skin. Euro Patent Applications. 13 pp.

Cuevas-Rodríguez EO, Yousef GG, García-Saucedo PA, López-Medina J, Paredes-López O, Lila MA (2010). Characterization of Anthocyanins and Proanthocyanidins in Wild and Domesticated Mexican Blackberries (*Rubus* spp.). J. Agric. Food Chem. 58:7458-7464.

Dencausse L, Nsourankoua H, Artaud J, Clamou JL (1995). Comparison of the lipidic compositions of butters of Pentadesma and the shea tree. OCL 2:143-147.

Di Vincenzo D, Maranz S, Serraiocco A, Vito R, Wiesman Z, Bianchi G (2005). Regional variation in shea butter lipid and triterpene composition in four African countries. J. Agric. Food Chem. 53:7473-7479.

Duke JA, Atchley AA (1986). Handbook of proximate analysis tables of higher plants Boca Raton Edition. CRC press, Florida, Boca Raton. 389 pp.

Greenwood M (1929). Shea nuts and shea butter. Bull. Agric Depart. 59-100.

Harlan J (1975). Crops and Man American Society of Agronomy, Crop Science Society of America, Madison. 295 pp.

Hutchinson J, Dalziel JM (1954). Flora of West Tropical Africa. Whitefriards Press Ltd., London. 11pp.

Kayodé APP, Linnermann AR, Nout MJR, Van Boekel MAJS (2007). Impact of sorghum processing on phytate, phenolic compounds and *in vitro* solubility of iron and zinc in thick porridges. J. Sci. Food Agric. 87: 832-838.

Mabberley DJ (1987). The Plant-Book, Cambridge University Press, Cambridge, UK. 112 pp.

Maranz S, Wiesman Z, Bisgaard J, Bianchi G (2003). Evidence for indigenous selection and distribution of the shea tree, Vitellaria paradoxa and its potential significance to prevailing parkland savanna tree patterns in sub-saharan Africa North of Equator. J. Biogeogr. 30:1505-1516.

Mathur PB, Rao JS, Vadez V, Sharma KK (2009). Transgenic Strategies for Improved Drought Tolerance in Legumes of Semi-Arid Tropics. J. Crop. Improv. 24: 92-111.

Natta A (2003). Ecological assessment of riparian forests in Benin: phytodiversity, phytosociology and spatial distribution of tree species. PhD Thesis, Wageningen University. 215 pp.

Natta A, Sogbégnon R, Tchobo F (2010). Endogenous knowledge and importance of Pentadesma butyracea (Clusiaceae) for the populations autochtones in Western North of Benin. Fruit Veg. Cereal Sci. Biotechnol. 4: 18-25.

Natta AK (2007). Restoration of riparian forests ecological diversity in landscape management plans: issues and lessons from Benin. Ecological Restoration of African Savanna Ecosystems. Proceedings of the third Regional Network for the Synergy Between the Conservation on Biological Diversity and the Convention to Combat Desertification in West and Central Africa (RNSCC). International Seminar, 6 February, Cotonou, Benin. CEDC - Cameroon /CML, Leiden University, the Netherlands. 156 pp.

Natta AK, Adomou AC, Tchabi VI, Sogbégnon AR, Mensah GA, Sinsin BA (2011). Inventory, typology and structure of natural populations of Pentadesma butyracea (Clusiaceae) along the Atacora chain in North-Western Benin. Anal. Sci. Agro. 70: 10-24.

Nkouam GB, Barth D, Dirand M, Tchatcheng JB (2007). Oil extraction from shea kernel (Vitellaria paradoxa, Gaertn.) and Canarium pulp (Canarium schweinfurthii, Engl) using supercritical CO2 and hexane: a comparative study. Res. J. Appl. Sci. 2:646-652.

Pietta P (2000). Flavonoids as antioxidants. J. Nat. Prod. 62:1035-1042.

Raponda-Walker, Sillans R (1961). Useful plants of Gabon. Paul Lechevalier, Paris, France. 614 pp.

Salazar FJ, Bolterman R, Hernandez I, Quesada T, Romero JC (1987). Role of prostaglandin and angiotensin II in ANP-induced natriuresis. Agents Actions Suppl. 22:11-23.

Singleton VL, Rossi JA (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am. J. Enol. Vitic. 16:144-158.

Sinsin B, Sinadouwirou T (2003). Valorisation socio-économique et pérennité du *P. Butyracea* Sabine en galeries forestières du Bénin. Cah. Agric. 12:75-79.

Sompong R, Siebenhandl-Ehn S, Linsberger MG, Berghofer E (2011). Physicochemical and antioxidative properties of red and black rice varieties from Thailand, China and Sri Lank. J. Food Chem. 124:132-140.

Tano-Debrah K, ohta Y (1994). Emzyme assisted aqueous extraction of fat from kernels of shea tree, Butyrospermum parkii. J. Am. Oil Chem. Soc. 9: 979-983.

Tchobo F, Natta A, Barea B, Barouh N, Piombo G, Pina M, Villeneuve P, Soumanou M, Sohounhloue D (2007). Characterization of *Pentadesma butyracea* sabine butters of different production regions in Benin. J. Amer. Oil Chem. Soc. 84:755-760.

Tchobo FP, Alitonou GA,, Noudogbessi JP, Laguerre M, Barea B, Georges P, Natta KA, Villeneuve P, Soumanou MM, Souhounhloue KCD (2013). Evaluation of the chemical composition of *Pentadesma butyracea* butter and defatted kernels. Int. J. Biosci. 3: 101-108.

Tchobo FP, Piombo G, Pina M, Soumanou MM, Villeneuve P, Sohounhloué DCK (2009). Enzymatic synthesis of cocoa butter equivalent though transesterification of *Pentadesma butyracea* butter. J. Food Lipids 16:605-617.

Wabo HK, Kikuchi H, Katou Y, Tane P, Oshima Y (2010). Xanthones and a benzophenone from the roots of *P. butyracea* and their antiproliferative activity Phytochem. Lett. 3: 104-107.

Yé GS, Destain MF (2004). Study of a press to oil:Technical characterization of the manual presses with shea tree existing in Burkina Faso and determination of the effort of crushing of almond of shea tree.Agronomic memory DEA. Sciences and biological Engineering. University faculty of Agronomic Sciences of Gembloux**.** 75 pp.

Zavrajnov AI, Nikolow DI (1990). Mechanization of the preparation and the forage conservation. ISBN-5-10-000761-3. 336 pp.

Zelefack F, Guilet D, Fabre N, Bayet C, Chevalley S, Ngouela S, Ndjakou-Lenta B, Valentin A, Tsamo E, Dijoux-Franca MG (2009). Cytotoxic and Antiplasmodial Xanthones from *P. butyracea*. J. Nat. Prod. 72: 954-957.